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Solubility Study of Albumin Solders for Laser Tissue-Welding

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Background/Objective: **Current albumin solders for tissue-welding are soluble in physiological fluids, prior to laser irradiation. These solders are therefore subjected to mechanical alterations, which can weaken the solder-tissue repair. In this study, an albumin solder (laser activated) was developed with low solubility and with the ability to retain (partially) its mechanical characteristics in saline solution.**
Study Design/Materials and Methods: **Gauged protein samples of solder were immersed into 0.5 ml saline solution for fixed intervals of time. The solder samples contained four Bovine Serum Albumin (BSA) concentrations: 56%, 66%, 70%, and 75% (by weight). A Bradford protein assay measured the BSA solubility of the solders. The 70% and 75% BSA solders were also used to weld in vitro Wistar rat intestine sections with a diode laser (λ = 810 nm, power = 270 mW).**
Results: **The solubility of the 75% BSA solder was significantly decreased with respect to the other solders (Anova, $P < 0.05$). This solder also showed comparable weld strength (13 gm) to the 70% BSA solder.**
Conclusion: **The 75% BSA solder strongly reduced the albumin solubility in saline solution, without affecting its tissue-welding properties. Lasers Surg. Med. 23:258–262, 1998.**

Key words: tensile strength; protein concentration; biomaterial; mechanical properties

INTRODUCTION

Current methods for surgical wound closure involve the approximation of tissue segments with sutures, clips, or staples. Although these techniques are generally reliable, there are a number of limitations (such as scar formation and foreign body reaction) that have prompted a search for alternative methods of wound closure [1–5]. A very promising approach is the use of protein tissue solders, which are activated by lasers. Among these biomaterials, the most popular are the albumin solders [5–8]. During laser tissue-welding, the solder is applied to the severed tissue and a laser irradiates the protein solder, which bonds the tissue. The bonding between the solder and the tissue allows the severed parts of the tissue to be approximated to help its repair process. Protein laser-activated solders are absorbable within a few days and therefore are less likely to cause scar formation. Also, laser-solder techniques are usually faster and easier than conventional closure procedures. Despite these advantages, current albumin solders (fluid and solid) are soluble in physiological fluids before laser irradiation [5–9]. The solder can often be subjected to blood dilution during operation, which weakens the repair tensile strength and change the mechanical characteristics of the solder. The overall result is an impairment of the reliability and reproducibility of the laser-solder technique.

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TABLE 1. Solder Solubility Data*

Solder [BSA] (g/g)	Preparation time (s)	T_{max} (°C)	n	Solution time (s)	Solder thickness (mm)	Solder weight (mg)	SR (μg/μg) %
56%	50	31 ± 1	9	45 ± 3	0.16 ± 0.02	1.4 ± 0.2	>36 ± 6
56% Heated	50	31 ± 1	3	48 ± 1	0.15 ± 0.01	1.5 ± 0.1	>34 ± 2
66%	90	37 ± 3	9	110 ± 3	0.16 ± 0.01	1.4 ± 0.3	18 ± 8
66% Heated	90	37 ± 3	3	109 ± 3	0.16 ± 0.01	1.4 ± 0.1	15 ± 3
70%	240	38 ± 1	9	299 ± 5	0.18 ± 0.02	1.3 ± 0.1	10 ± 3
75%	480	42 ± 2	9	3000 ± 60	0.18 ± 0.02	1.4 ± 0.1	6 ± 4

*Solder [BSA], BSA concentration of the protein foils (weight/weight); Preparation time, time required to homogenize by compression the solder; n, number of paired protein foils in saline solution for each BSA concentration; solution time, time interval for the protein foils dilution; SR, solubility ratio of the protein foils.

We have developed a novel albumin biomaterial, which successfully welded tissue, had low solubility, and preserved partially its mechanical characteristics in physiological fluids.

MATERIALS AND METHOD

Solder Preparation

Lyophilized BSA was reconstituted with distilled water in four different concentrations, namely, 56%, 66%, 70%, and 75% (weight/weight). The solder components were homogenized by mechanical compression with a specifically manufactured compressor. A type K thermocouple (diameter ~ 0.5 mm) was connected to a digital multimeter and it was inserted into the solder to measure the peak temperature during the BSA reconstitution. No sting tools (such as needles) were used because they were able to mix BSA and water at concentrations < 70% but they could not homogenize by compression BSA and water at higher concentrations (⩾ 70%) [8,9]. The glue was solid and malleable until it dried up and became rigid. Before dehydration, the solder was reduced to a fixed thickness film with a parallel plate vice. Rectangular portions of the protein film (foils) were cut with a surgical blade and they were weight by a digital balance (s.d. = ± 0.1 mg). These protein samples were prepared with fixed weight (1.4 ± 0.2 mg) and thickness (0.17 ± 0.02 mm) to standardize the solder dimensions in solution (Table 1). The solder thickness was measured with a digital caliper (s.d. = ± 0.01 mm). After preparation, the solder samples were used for the protein assay.

Protein Assay

A total protein assay (Bradford) was performed to measure the solubility of the solder with different BSA concentrations. The standard BSA concentrations, used in this assay, were 75, 100, 250, 500, and 1,000 μg/ml in 0.9% NaCl solution (Fig. 1); 300 μl of Coomassie reagent were added to 10 μl of the unknown and standard samples with a 150 μl multiple pipette in two steps. The blanks consisted of 10 μl saline solution and 300 μl of reagent. The samples were prepared as follows: two foils of solder from each BSA concentration were placed in 0.5 ml of saline solution for a fixed amount of time (varying between 45 sec to 50 min). During this period, the solution was shaken every minute for 2–3 sec to prevent protein saturation at the bottom of the 1.5 ml Eppendorf tube. The solder dilutent was then extracted with a pipette and placed in a separate Eppendorf tube. The solder samples, which were not dissolved after the solution time, were rescued and observed under a dissecting microscope (~×50). Ten microliters from each solder unknown

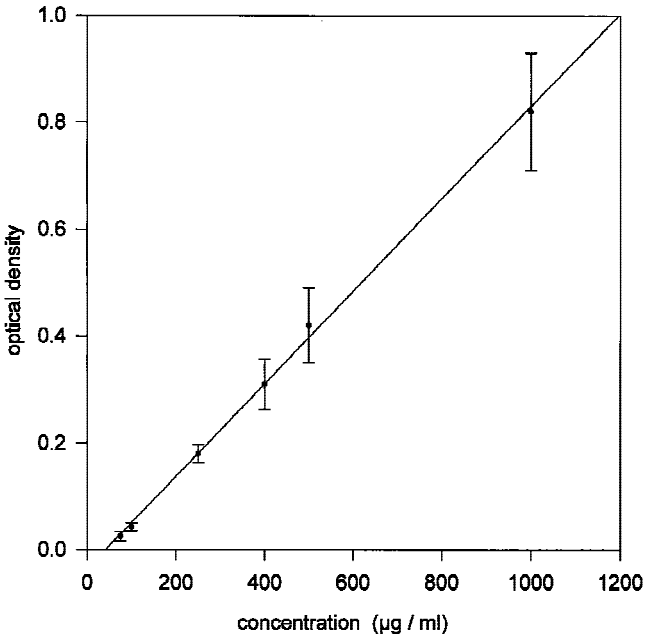

Fig. 1. Optical density curve of the Bradford Assay using BSA standard concentrations (75–1,000 μl/mg).

solution were transferred in a well of a 96-well plate, which was scanned by a plate reader. The Bradford assay used a Coomassie E Brilliant Blue G-250 dye, which generated a blue color when bonded to BSA. Therefore the plate was read by the spectrometer at 595 nm. Three sets of paired solder samples were used for each BSA concentration. The standards, unknowns, and blanked wells were done in triplicates and three independent experiments were performed.

Laser Tissue-Welding

Laser tissue-welding of Wistar rat's intestine was performed by using the solder to assess and compare the soldering properties of the 75% BSA solder with respect to the lower concentrated solders. A diode laser was used during the experiment (λ = 810 nm, power = 270 mW in continuous wave). The laser was coupled with an optical fiber (core diameter = 400 μm), which allowed the surgeon to delivered precisely the laser beam to tissue. The surgical procedure was performed under an operating microscope (×40 times). The 70% BSA solder was chosen among the lower concentrated solder because it created stronger tissue welds [9]. The solders contained also Indocyanine Green (0.25% weight/weight) to absorb the laser infrared radiation [10]. Three centimeter long sections were dissected from the small intestine of the animals and cut in two equal parts. The two tissue segments were approximated end-to-end and one or two rectangular protein foils of solder ($\approx$ 0.6 × 6 mm) were positioned over the anastomosis site. These solders were irradiated by the laser, which was absorbed by the IG dye contained in the solder. The photothermal process, induced by the dye absorption, caused the solder foils to bond the intestine, joining the two tissue sections. The tissue around the solder foils was kept moist and the excess water was carefully absorbed with gauze before laser irradiation to avoid dilution of the 70% BSA solder. This was done to ensure ideal soldering conditions during laser tissue-welding. The laser power, radiation dose (energy/solder weight), and the solder dimensions (surface area and thickness) were kept constant throughout the operations [9].

The soldered intestine was tested acutely to assess the tensile strength of the repair by using a calibrated tensiometer (Instrom Mini 55, MA), interfaced with a personal computer. The specimen was clamped to the tensiometer by pneumatic grips, which moved 22 mm/min until the solder weld failed, by separating the two intestine segments. The tissue was kept wet throughout the procedure, since with drying the tensile strength increased [11]. The breaking force was recorded.

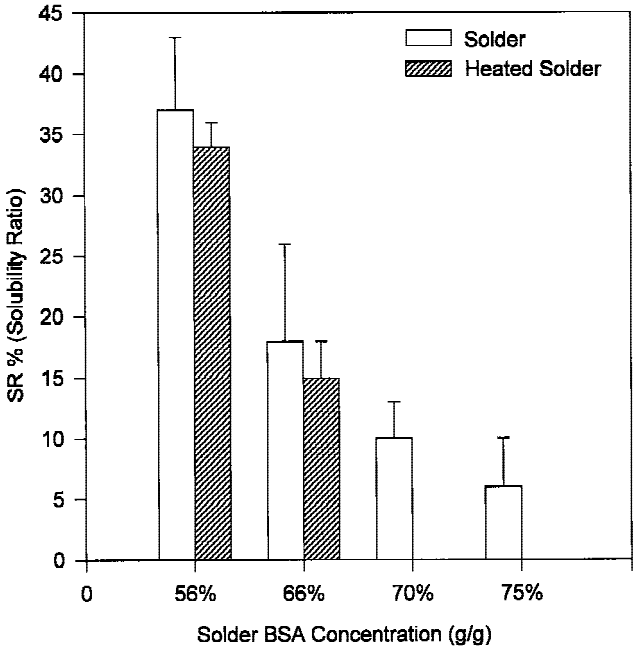

Fig. 2. The older "Solubility Ratios" (SRs) in percentage are given vs. the BSA concentrations (56–75%) of the solder (mean ± standard deviation). The dashed columns represent the 56% and 66% solders, heated at 50°C for 15 minutes.

Statistical Analysis

Statistical comparison of means was made by using the Student's *t*-test for unpaired observations and the one way ANOVA (0.05 level of significance). The linear regression and histograms were generated by Sigma Plot (2.01 version).

RESULTS

Solder solubility

The ratio between the weight of BSA in solution (measured by the spectrophotometer) and the two correspondent protein samples (Solubility Ratio, SR) was chosen to measure the solder solubility (Fig. 2). The SR was preferred to the ratio between the dissolved BSA and the BSA content of the protein sample because the whole solder foil is relevant for repair strength, during laser tissue-welding.

The SRs of the different concentrated solders were significantly different (Anova, $P < 0.05$). The solder with 56% BSA content dissolved > 37% in

45 sec. In this case, the optical density of the wells exceeded the linear range of the protein assay (75–1,000 μg/ml). The solder was also observed microscopically to be completely in solution after 3 min. The 66% solder dissolved 18% in 110 sec. The solder was observed to loose shape in its curling and folding after 45 sec. These samples appeared under the microscope to be swollen and to break easily, when they were pulled apart with fine forceps. The 70% solder dissolved 10% in 299 sec. This solder was observed to twist and curl after 85 sec, nevertheless its rectangular shape was still recognized after the solution time. These samples appeared under the microscope to be swollen, very soft, and to break easily when they were pulled apart with fine forceps. The 75% solder dissolved 6% in 50 min. The solder was swollen but retained its shape throughout the dilutent immersion and showed only a slight twist. These samples reduced their original rigidity but they appeared to resist the forceps stress (before breaking), when they were pulled apart (Table 1).

The maximum temperature reached during the solder preparation was 41°C for the 75% solder and its preparation time was 8 min (Table 1). Since the preparation time and temperatures of the other solder samples were significantly lower (30–35°C), additional solder samples with 56% and 66% BSA content, were heated in a dry bath at 50 ± 2°C for 15 min before undergoing the protein assay described above (Table 1). This was done to assess the effect of the increased temperature on the solder solubility. The solder solubility after the thermal treatment was not significantly different ($P > 0.05$) respect to the not heated solder (Table 1 and Fig. 2).

Laser Tissue-Welding

The protein solder joined all the specimens successfully and the average tensile strength of the soldered tissue ranged between 6 and 13 gm, for both 70% and 75% BSA solders (Fig. 3). There was no significant difference between these means when compared with the Student's *t*-test. In both groups, the repair strength was doubled when two protein foils were welded across the intestine anastomosis instead of one. In all cases, the weld rupture was due to the failure of the solder-tissue interface [9]. These results are summarized in Table 2.

DISCUSSION

The increase of BSA concentration resulted in a decrease of the solder solubility. The decrease

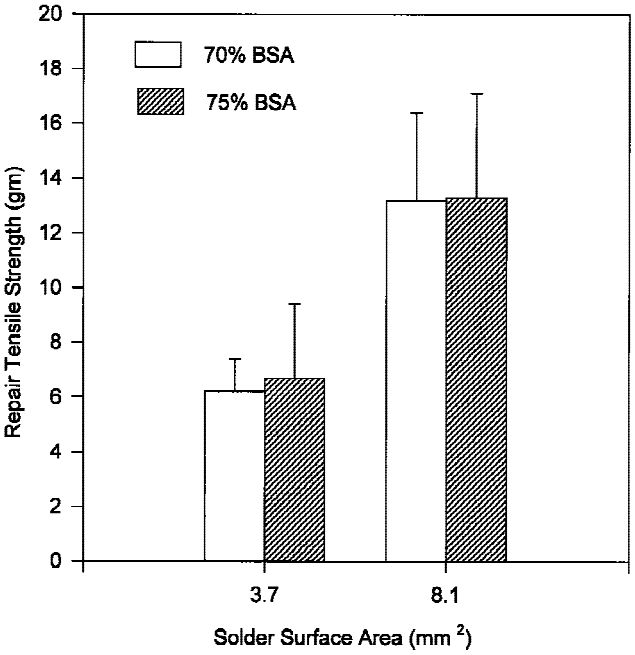

Fig. 3. The tensile strength (mean ± s.d.) of the laser anastomosis is shown on Y-axes, for the 70% and 75% BSA solders. The solder surface area relative to the weld tensile strength is displayed on the X-axes.

of solubility was significantly higher for the solder with 75% BSA content than for the lower concentrated solders. For this reason, it was impossible to choose a fixed time interval to dilute the solder with different BSA concentrations. The SR was 6% after 50 min. solution time for the 75% BSA solder, while the SR of the 70% BSA solder was 10%, after only 5 min solution time. The water solution process altered the mechanical properties of the solder, such as shape and rigidity. The shape and rigidity of the 56% and 66% solders were soon (45 sec) altered in saline solution by curling, swelling and dissolving. The 70% solder twisted and bent after 85 sec despite its solubility was less than the 56% and 66% solder solubility. After 5 min in solution, these samples appeared under the microscope, swollen and much softer than before being diluted. Also, they could be easily broken if they were pulled apart with fine forceps. The 75% solder, on the other hand, did not lose its rectangular shape during the 50 min dilutent immersion; it only developed a light twist without bending. This solder appeared under the microscope to be swollen and flexible. It was softer than before being immersed, but it appeared to oppose resistance when it was pulled apart. The less concentrated solders (56% and 66%) did not changed their solubility ratio after

TABLE 2. Tissue Repair Data*

BSA% (g/g)	n	Solder weight (mg)	Solder area (mm^2)	Laser power (mW)	Laser dose (J/mg)	Weld strength (gm)
70 (1 foil)	4	0.62 ± 0.05	3.8 ± 0.5	270 ± 20	22.6 ± 2.6	6.2 ± 1.2
75 (1 foil)	4	0.60 ± 0.08	3.6 ± 0.5	270 ± 20	22.8 ± 1.7	6.7 ± 2.7
70 (2 foils)	4	1.35 ± 0.06	8.2 ± 0.6	270 ± 20	20.7 ± 0.8	13.2 ± 3.2
75 (2 foils)	4	1.32 ± 0.09	8.0 ± 0.6	270 ± 20	21.7 ± 0.9	13.3 ± 3.8

*The laser parameters and solder characteristics (mean ± SD) are given for the tissue welding of the rat intestine. One or two albumin foils with 70% and 75% BSA content were applied onto the tissue. The foil thickness was constant (0.15 ± 0.01 mm) during all surgical procedures. BSA%, BSA concentration of the protein foils; n, number of laser-solder repairs; Solder area, averaged solder surface area in contact to the intestine during laser welding; Laser power, laser power during solder welding; Laser dose, averaged laser dose used for solder welding; Weld Strength, breaking force of the solder-tissue weld.

being heated at 50°C for 15 min. Therefore, the temperature difference of the solder preparations at different BSA concentrations did not influence the solder solubility, which depended only on the BSA concentration. The 75% BSA solder showed comparable tensile strength to the 70% BSA solder (when it was bonded to the rat small intestine), but the 75% solder better preserved its shape and rigidity in saline solution. The higher solubility of the 70% BSA solder did not decrease its tensile strength, presumably because the laser welding procedures were performed in dry ideal conditions. The soldered tissue failed under stress because of interface rupture (100% of specimens), which indicated the rigidity of the irradiated solder was stronger than the solder-tissue weld. The novel method of increasing the BSA concentration (> 70%) of the solder determined a dramatic decrease of the solder solubility prior to laser irradiation, without affecting its welding properties. The insolubility is an important characteristic of the solder for its clinical applications. Indeed, physiological fluids and blood hemorrhages might dissolve or significantly degrade the solder before laser tissue-welding. This would alter the mechanical properties and the tensile strength of the welds, compromising and impairing the repeatability of the surgical procedure. A potential advantage of the "insoluble" solder is that it may be filled with drugs (Growth Factors, for example) and it may serve as a drug delivery system to guide and help the tissue healing, after laser welding [12]. The low solubility solder may preserve efficiently (without significant dilution) such drugs before laser irradiation, and it may deliver them subsequently. Further investigations are needed to test the 75% BSA solder in vivo (not in dry ideal condition) and to clarify the physical-chemical process responsible for the solder insolubility.

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